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Research Article

Therapeutic Response of Human Occipital, Breast and Oral Cavity Tumors to Paclitaxel in NOD SCID Mice

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Abstract

Preclinical chemotherapeutic drug screening is performed using cultured tumor cells or human tumor xenografts. However, direct correlation is not established between the response of mouse and human patients. Factors like tumor size at which treatment is initiated, schedule and doses of treatment etc. are important for the success of such screenings. The present study was undertaken to assess *in vivo* response of squamous cell carcinoma of occipital region, carcinoma of breast and squamous cell carcinoma of oral cavity collected from the operation theater of ACTREC to Paclitaxel. Pieces of tumor from occipital and oral cavity were grown subcutaneously in NOD SCID mice whereas breast tumor was grown orthotopically near the mammary fat pad. Paclitaxel treatment was given at the dose of 25 mg/kg body weight on 1, 5 and 9th day after the tumor attained the size of 50-300 mm^3. Treatment group animals showed significant tumors growth delay by 12 weeks. Occipital tumor group showed significant growth delay as well as anticancer activity when maintained for extended period of 22 weeks whereas breast and oral cavity tumor did not show anticancer activity when maintained for 19-20 weeks. The results of the present study warrant systematic study using varying doses and/or duration of treatment.

Keywords: Human Tumor Xenograft, Paclitaxel, Treatment.

Introduction

Cancer is a major health problem in most parts of the world. Basic research and clinical trials on cancer patients are essential components of the cancer drug discovery process. However, animal experiments form the basis of these trials. Use of murine tumor in a syngenic animal model offer several advantages. They are easily available, low cost, have a long history of use, have a strong baseline of reproducible drug response data and studies can be easily conducted using statistically significant numbers. The disadvantages are that the tumor cells are rodent origin, express the mouse/ rat homologues of the desired targets and the tumor grows pretty fast. Relevance of the model depends on how best it replicates the original histology, physiological effects, biochemical pathways and metastatic pattern observed in the same human tumor type. Artificial features like host origin blood supply and neovascularization; murine stroma; and technical difficulty in orthotopic transplantation influence the outcome of their use. These tumors have lower acceptance rate, are often encapsulated, rarely infiltrate/ metastasize and do not always reflect the clinical situation.[1,2] Still, tumor growth induction under the subcutis has been quoted as a model by several authors. Results of use of such models may not be absolute predictive of the drug activity.[3]

Earlier, antitumor activity in murine ascitic leukemia models was assessed on the basis of percent mean or median increase in life span, net log_{10} cell kill, and long-term survivors of the mice. Treatment with test agents can be initiated either before tumor development or after a tumor growth appears. If the treatment begins the day after or on the day of tumor cells/ tissue implantation, the experiment is considered as a '*tumor growth inhibition study*'. If treatment begins when an established tumor nodule (50-200 mm^3) is present, the experiment is considered as a '*tumor growth delay study*'. Activity in a tumor growth delay shows a stronger evidence of clinical potential than activity in a tumor growth inhibition.[4] One of the requirements of these assays is that drugs be administered at doses producing tolerable normal tissue toxicity, so that the response of the tumor to the treatment can be observed for a relatively long period of time.

Based on the above information, the present study was undertaken to test the response of human tumor to standard anticancer drug, Paclitaxel. Identification and isolation of active ingredients, taxol, from the tree of *Taxus brevifolia* and its antitumor activity was reported *in vitro* for P388 cell line for the first time by Wani et al., 1971.[5] Taxol is a microtubule stabilizing agent which promote the

stabilization of microtubule polymerization interrupting the process of cell division and promoting apoptosis.[6] Paclitaxel has antitumor activity against breast tumor, head and neck cancer and sarcoma.[7] Paclitaxel is also promising agent against previously treated breast cancer cases.[8] We evaluated the therapeutic activity of Paclitaxel against three human tumors (occipital, breast and oral cavity) transplanted in the SCID mice.

Materials and Methods

Animals and their maintenance:

NOD SCID mice of 6-8 weeks age were procured from the Animal Facility of the ACTREC, Navi Mumbai, for the experimentation. The experimental protocol was approved by the Institutional Animal Ethics Committee of the ACTREC which is registered with the CPCSEA, Ministry of Environment and Forests, Government of India for breeding and conducting experiments on small laboratory animals. The animals were housed in groups of 4-5 animals per polysulphone make individually ventilated cages under controlled conditions of 55 ± 5% humidity, 23 ± 2°C temperature, and 12-hr light/12-hr dark cycle under specific pathogen-free conditions. All mice were maintained on sterilized corn cob bedding material and in-house pelleted animal feed and UV treated water *ad libitum*. All mice were handled for experimentation in a cage changing station with sterile technique.

Human tumor samples and their implantation in the mice:

Freshly operated human tumor samples were collected in 15 ml capacity sterile tubes containing plain RPMI 1640 medium (Invitrogen, USA) with antibiotics through the Biorepository Laboratory of the ACTREC, Navi Mumbai, which has obtained these samples after obtaining due informed consent of the patients from the operation theatre of the ACTREC. NOD SCID mice were anaesthetized by 2% isoflurane gas to achieve the surgical anesthesia by using the isoflurane gas anesthesia assembly (VetEquip, USA). Small pieces of approximately 5x5 mm size histologically viable tumor tissues collected as above were washed in fresh RPMI 1640 medium containing antibiotic. Connective, necrotic or suspected necrotic tissue as well as blood clots, if any, was removed by use of sharp scalpel blade and then rinsed three times using RPMI 1640 medium. For occipital tumor (squamous cell carcinoma) and oral cavity tumor (moderately differentiated squamous cell carcinoma), small skin incision was made at the midline back region and single piece of occipital or oral tumor was implanted aseptically in 10 and 14 NOD SCID mice, respectively, under the subcutis at the flank region of anaesthetized NOD SCID mice of either sex. In case of breast tumor (sarcomatoid carcinoma), skin incision was made at the flank region and single piece of tumor tissue was orthotopically implanted near the mammary fat pad of 12 female NOD SCID mice. Skin wound was sealed with the help of sticking glue, Vetbond (3M, USA) as described earlier.[9]

Tumor growth measurement and Paclitaxel treatment:

Tumor growth was measured with the help of electronic vernier caliper (Mitsuyoto, Kawasaki, Japan). Control group animals were maintained without any treatment. Treatment group animals were injected with Paclitaxel (Dr. Reddy's Laboratories, Hyderabad, India; Proprietary name- Mitotax) at the dose rate of 25mg/kg body weight of the animals diluted four times with the normal saline. Paclitaxel was injected on 1, 5, and 9th day starting after the tumor reached 50-300mm^3 size which occurred on 5-7th day after transplant.[6,10,11] Subsequently, tumor size was measured every week for next 12-22 weeks with the help of vernier caliper. Tumor volume was calculated in cubic mm using the formula $(W^2xL)/2$, where W= tumor width and L= tumor length in mm. Data was assessed using the line graph. Anticancer drug effect was assessed as ineffective when the tumor started growing beyond 500 or 1000mm^3.4

Statistical analysis:

Data of differences between response of occipital, breast and oral cavity tumor to the standard anticancer drug, Paclitaxel was analyzed for significance using student's't' *test*. A probability value was deemed statistically significant if $p < 0.05$.

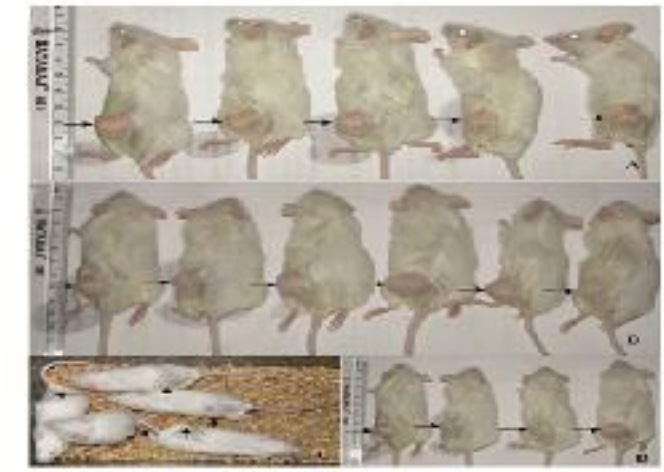
Results

Occipital tumors (squamous cell carcinoma) grown in NOD SCID mice responded well to the standard anticancer treatment of Paclitaxel at the rate of 25mg/kg body weight of the animals when compared to the control group. Response of occipital tumors to Paclitaxel is shown in Table.I. Control group animals of occipital tumor group had to be sacrificed on 12th week from the date of transplant because the tumor had reached to an average size of 1900mm^3 (Fig.1A) whereas treatment group animals showed below 30 mm^3 size of tumor at 12th week. *In vivo* results of the response of occipital tumor to Paclitaxel treatment are depicted in Fig.2A. Since the tumor sizes in case of occipital tumor treatment group animals were significantly low, the last animal of this group was maintained up to 22 weeks from the date of transplant. The tumor sizes in treatment group animals were below 50 mm^3 even at 22nd week from the date of transplant.

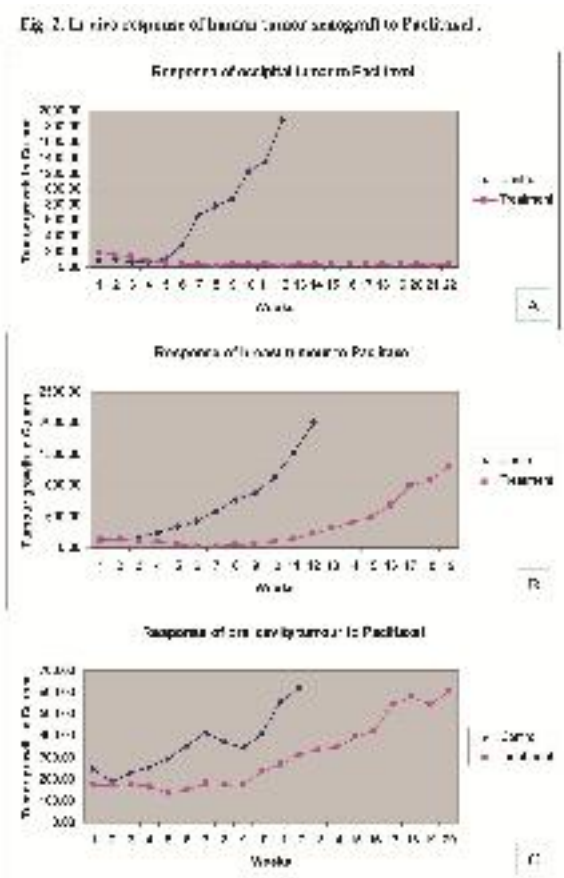
Table.1: Response of three different tumors to Paclitaxel.

	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week	9th week	10th week	11th week	12th week
Occipital tumor												
Control group	83.36 ±33.21	103.56 ±61.59	59.79 ±29.77	70.29 ±39.30	115.45 ±31.045	285.89 ±136.01	668.41 ±206.29	794.62 ±206.39	876.48 ±171.98	1235.14 ±284.06	1359.61 ±349.44	1886.49 ±288.75
Treatment group	192.77 ±52.97	163.07 ±38.55	130.90 ±39.88	82.47 ±46.43	64.94 ±34.66	43.13 ±34.46	37.72 ±25.88	24.20 ±17.88	33.28 ±20.51	33.28 ±20.51	33.28 ±20.51	26.97 ±18.36
Breast tumor												
Control group	120.77 ±15.34	145.07 ±27.18	172.02 ±36.69	251.95 ±86.32	332.04 ±192.21	430.12 ±252.99	593.32 ±330.69	775.76 ±398.16	879.30 ±416.97	1120.95 ±532.02	1531.38 ±849.42	2013.21 ±1074.8
Treatment group	117.52 ±18.52	135.12 ±56.14	112.15 ±30.33	99.78 ±57.19	64.00 ±65.54	22.18 ±24.73	33.18 ±60.83	41.81 ±78.08	56.99 ±75.32	86.65 ±88.87	137.54 ±101.76	228.36 ±164.60
Oral cavity tumor												
Control group	246.44 ±91.59	184.18 ±54.97	227.70 ±72.38	253.31 ±119.98	294.06 ±211.85	354.60 ±245.58	416.05 ±273.04	370.82 ±111.99	345.01 ±71.93	406.06 ±142.05	558.69 ±213.96	624.19 ±275.70
Treatment group	172.65 ±37.81	172.01 ±33.97	176.49 ±32.02	168.18 ±38.97	134.14 ±33.50	153.38 ±24.05	181.65 ±35.71	174.03 ±48.44	178.15 ±71.81	233.85 ±96.10	270.01 ±170.84	316.54 ±224.44

Tumor size values of control and treatment groups are mean ± SD.



Control group animals of breast tumor (sarcomatoid carcinoma) group reached to an average tumor size of more than 2000 mm^3 on 12th week from the date of transplant and therefore had to be sacrificed (Fig. 1 B). Human breast tumor (sarcomatoid carcinoma) grown in NOD SCID mice responded well to the standard anticancer treatment of Paclitaxel at the rate of 25 mg/kg body weight of the animals when compared to the 12th week values of control group (Fig.1C). The tumors in treatment group animals gained momentum and started growing beyond 500 mm^3 after 15 weeks from the date of tumor transplant. *In vivo* results of the response of breast tumor to Paclitaxel treatment are depicted in Fig.2B. Since the tumor growth in treatment group animals was substantially low, the animals were maintained till 19th week from the date of transplant. Tumor size in treatment group animals was below 500 mm^3 up to 15th week from transplant. However, after 15th week the tumor grew significantly and reached the size of ~1300 mm^3 at 19th week (Fig.1D).



Human oral cavity tumor grown in NOD SCID mice also responded well to the standard anticancer treatment of Paclitaxel at the rate of 25mg/kg body weight of the animals when compared to the 12th week values of control group. Response of oral cavity tumors to Paclitaxel is given in Table.1. The control group animals had tumor size of 624mm^3 at 12th week as against the treatment group animals which had tumor size of only 317mm^3. However, the tumors from treatment group animals did not grow beyond 600 mm^3 even when maintained for 20 weeks after transplant. *In vivo* results of the response of oral cavity tumor to Paclitaxel treatment are depicted in Fig.2C.

Discussion

Tumor cells grow and divide faster than non-malignant cells. Anticancer drugs have tendency to be more effective against rapidly growing and dividing cells than quiescent cells. This is the basis of treatment of malignancy with cytostatic and cytotoxic agents. Dividing rate of all tumor cells varies. In addition, chemotherapeutic response of all tumor cells to anticancer drugs may also vary. Therefore, it makes difficult to kill the tumor cells at the same rate. Cisplatin, taxol, and doxorubicin are few examples of routinely used anticancer drugs to treat many human cancers.[12]

Taxol is an excellent water soluble drug which exhibits antitumor activity against variety of tumors such as ovarian cancer, breast cancer, head and neck cancer, non-small cell lung cancer, and sarcoma. However, Taxol is reported inactive against the hepatocellular carcinoma and intra-peritoneal SKOV3 human ovarian cancer. Paclitaxel is also reported inactive in reducing the incidence of lung metastases from orthotopically implanted MDA-MB-435 human breast cancer.[13] To confirm the previous reports of wide spectrum anticancer activity of Taxol, locally collected human tumor samples from the operation theater of ACTREC were subjected to test their response to this drug.

Taxol is a clinically potent and promising microtubule-targeting agent for the treatment of cancer. However, resistance to Paclitaxel remains a limiting factor to its efficiency in treating the patients. Despite this limitation, Paclitaxel is at the frontline of cancer therapy and has posed the challenges to understand the molecular mechanisms of Paclitaxel resistance.[10] Traditional methods employed for testing the potential efficacy of chemotherapeutic drugs are useful to evaluate the response of human tumor cell lines and xenografts to the test drug of interest. Dose selection of this study was based on the previous reports of *in vivo* anticancer activity of Taxol (Chou et al, 1998; Chou et al., 2008; Trail et al., 1999).[6,14,15] In the present study occipital, breast and oral cavity tumors responded significantly to the treatment of Paclitaxel when compared to the control group values up to 12th week from the date of tumor transplant (Fig. 2. A, B and C). In case occipital tumor, the treatment group animals showed less than 50 mm^3 even when the animals were maintained up to 22nd week from the date of transplant. The treatment group animals would have disease free survival if maintained for another few weeks/months after 22 weeks. This suggested that the occipital tumor responded well to the Paclitaxel treatment and showed significantly low tumor growth as compared to the control group animals. Paclitaxel treatment also showed extended tumor-free life of the animals when compared with the control group animals. The tumor microenvironment is complex and consists of various types of stromal components that have an important role to support the tumor growth. Microenvironments of the normal cells are different from that of the tumor cells.[2] Occipital tumor might have not received required microenvironment in the present study and therefore it might have been possible for the Paclitaxel to exhibit complete anticancer activity.16 In case of breast and oral cavity tumor, data suggests that the treatment has its effect up to 15th and 16th week of treatment, respectively, but lost its effect over the tumor thereafter. All animals in control groups of occipital, breast and oral cavity tumor types were maintained for 12 weeks only.

In fact, Paclitaxel is a routine drug used for the treatment of breast cancer, ovary, lungs as well as head and neck cancers in human beings.[11] However, in the present study, breast and oral cavity tumor did not show expected anticancer activity to Paclitaxel when maintained for 19 and 20 weeks, respectively, after the date of tumor transplantation suggesting its substantially different antitumor spectrum from occipital tumor. Based on the type of tumor line, different sensitivity of Paclitaxel is well documented in the literature.[8] Traditionally, the anticancer treatment is largely focused on the cell of origin of the tumor and less on the microenvironment that supports the tumor growth. Animal models which use orthotopically implanted syngenic tumors are reported to be more predictive of responses than ectopic tumors.[12] The extrinsic factors also include immune cells, hormones, tissue stroma, growth factors, extracellular matrix, and angiogenesis. Schedule as well as sequence of drug combination might also be affecting the therapeutic outcome. Furthermore, the role of the ER, PR and HER2 status of breast cancer in anticancer activity also need to be explored.

Tumor dormancy is a well-known clinical phenomenon. In case of breast and oral cavity tumor, it is likely that despite treatment few cells remained viable but in dormancy and might have began to grow again after a relatively short lag and proliferated at the same average rate as tumor cells in untreated control group mice.[2,17] In order to achieve the complete cure from the disease, malignant tumor cell killing rate of the drug need necessary to be faster than that of the proliferation of the survived tumor cells.

Observations of metastases are especially important in such studies because they are the main determinants of the clinical course of the disease and

patient survival, and are the target of systemic therapy. It has also been reported that molecular pathways that promote tumorigenesis also promote metastases.[2] Surprisingly, in the present study we have observed that few mice from treatment group of occipital, breast as well as oral cavity tumor have developed metastasis to lungs as diagnosed by PCR method using human specific primers18 suggesting that either the dose or schedule of treatment is insufficient to kill the tumor cells. In case of breast tumor patients, it is important to know the previous exposure of the patient to the estrogen or chemotherapy. In case of metastatic model what is important is the magnitude of the benefit observed in mice, both in terms of the degree of tumor responses and overall survival. In such circumstances, survival is used as an endpoint of the response of the drug.[3]

The complex cellular communication with the mammary tissue and oral cavity microenvironment is surely deficient in this model because of the non-natural site of the tumor implant. Probably the normal *in vivo* tissue microenvironment must be playing a role in susceptibility to various drugs.[12,19] Tumors which acquire metastatic potential may pose more challenges to treatment because of the spread of the disease to distant organs of the body thereby increasing the effect of the disease on the host. In case of breast as well as oral cavity tumor transplanted mice, Paclitaxel treatment significantly prolonged the survival time of the mice as the tumor took long time to reach the volume to that of the control group mice. Similar results are reported by Chou et al. 2008[6] for the breast cancer xenografts in immuno-compromised models. Resistance of the breast cancer cell lines to Paclitaxel *in vivo* is also reported by Nakayama et al., 2009.[20] The development of resistance of the cancer cells to chemotherapy also depends partly on the genetic instability, heterogeneity and high mutational rate of tumor cells.[21]

Conclusion

In the present study, Paclitaxel showed significant antitumor effect on breast and head and neck cancer up to 12 weeks of transplant but did not show consistent anticancer effect when the animals were maintained for 19 and 20 weeks, respectively. However, taxol in combination with other drug may have greater synergistic anticancer effect.[8,22,23]. Better anticancer activity *in vivo* could be achieved by changing the treatment schedule and/or prolonging the duration of treatment. Treatment regimens with combination of multiple cytotoxic drugs from different companies with different mechanisms of action substantially improve the therapeutic efficacy as compared to either drug alone. Such additional studies with selection of optimum drug combination are required to fully understand the effect and mechanism of anticancer activity. They are also useful for conventional chemotherapy which may provide a promising therapeutic option for treating patients with advanced disease as well as metastasis.

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